Tier II Summary (PMRA #2181218, MRID 48574766) revised by the regulatory authorities, April 2013

Note that the reviewing agencies have different conclusions with respect to the interpretation of the lung tumors in female mice (see section IV – Evaluation, Summary and Conclusions).

Study Type Carcinogenicity study in the mouse

IIA 5.5.3 – MRID 48574766, PMRA 2181218

Report:	Kaiser St. (2011) MCW-2 TECH: 78-Weeks Oncogenicity (Feeding) Study in CD-1 Mice. Harlan Laboratories Ltd., Switzerland; unpublished report No. B80190, dated 31 August 2011. Sponsor reference No. R-23354 Dates of experimental work: (in-life phase) 26 February 2008 to 4 September 2009
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD No. 451, EEC Method B.32, and OPPTS 870.4200 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In an oral oncogenicity study (MRID 48574766; PMRA 2181218), 4 groups of 50 male and 50 female CD-1 mice (allocation A) were treated for 78 weeks with 0, 30, 200 or 1200 ppm of fluensulfone (MCW-2 tech., 96.7-98.65%, Batch No. 36372130-291-PF1) in their feed. Additionally, 6 males and 6 females (allocation B) were exposed to the same dietary concentrations and used for liver enzyme determination after 13 weeks of treatment. The average daily intakes of test material were 0, 4.2, 27.6 or 152.3 mg/kg bw/day for males and 0, 6.4, 39.0 or 188.4 mg/kg bw/day for females.

After 13 weeks of treatment, fluensulfone caused a slight decrease of hepatic alanine aminotransferase (ALAT) activity in females from the 1200 ppm dose group, but no change in ALAT activity was recorded after 78 weeks of treatment. In addition, after 13 weeks a weak increase of microsomal cytochrome P450 content was recorded, accompanied by a slight increase

in the activity of CYP4A-1 and CYP1A-1 in females only, and slight to moderate inductions of phase II enzymes uridine diphosphoglucuronosyl-transferase, glutathione S-transferase and epoxide hydrolase, mainly in females. Most of these changes occurred at 200 ppm and above, but were considered to be adaptive; none of these changes were considered adverse.

Treatment of CD-1 mice with fluensulfone at dietary concentrations of 30, 200 or 1200 ppm for up to 78 weeks had no effect on survival, clinical signs, or presence of palpable nodules and masses. Absolute food consumption (g/animal/day) was statistically significantly reduced at most weighing intervals in both sexes at 1200 ppm. The overall mean for animals treated with 1200 ppm compared to controls was 17% lower in males and 24% lower in females. In males, relative food consumption (g/kg bw/day) was not affected by the treatment. However, in females, relative food consumption was statistically significantly reduced at most weighing intervals at 1200 ppm and the overall mean over treatment was reduced by 21%. Cumulative body weight gain was decreased by 13% and 37% for males at 200 and 1200 ppm, respectively, and by 30% for females at 1200 ppm.

Absolute mean body weight and body weight gain were statistically significantly decreased in a dose-dependent manner in males at 200 and 1200 ppm (statistically significant from week 5 through 54 and throughout the study, respectively), and in females at 1200 ppm (statistically significant from week 2 onward for most measurements). The mean body weights at the end of the treatment period were reduced by 6% and 17% in the males of the mid and high dose groups, and by 12% in the females of the high dose group.

After 52 weeks, hematology revealed a slightly decreased absolute red blood cell counts in mice of both sexes at 1200 ppm (5% below controls) and decreased total white blood cells count in females only, accompanied by reductions in absolute neutrophil, eosinophil, lymphocyte and monocyte counts (19-25%). Total white blood cells and absolute neutrophil and eosinophil counts were also reduced in females at 200 ppm (19-43%). None of these changes was still present at terminal sacrifice after 78 weeks. Slight increases in serum ALAT and ASAT were noted in females from the 200 and 1200 ppm dose groups at week 78, and increases in SDH were noted in both sexes at 200 and 1200 ppm at 78 weeks (these parameters were not assessed at week 52).

Absolute and relative (to brain weight) prostate weights were observed in males in the 200 and 1200 ppm dose groups. Changes in other organ weights at 1200 ppm (increased relative kidney, adrenal gland, epididymal, testes, heart, and brain weight in males; decreased absolute kidney weight in females, and increased relative liver weight in females) were likely secondary to the effect of fluensulfone exposure on body weight.

Non-neoplastic lesions consisted of a statistically significantly increased incidence of bronchiolization (i.e. a change from flattened epithelium to cuboidal epithelium) in the lung of males and females treated at 200 and 1200 ppm (from control to high dose respectively, 1, 0, 24 and 31 in males and 5, 7, 43 and 48 in females; n = 50 all groups). Transmission electron

microscopy (TEM) analysis revealed hypertrophy of the epithelium of the terminal bronchioles affecting mostly the non-ciliated Clara cells as well as the directly surrounding ciliated cells.

The LOAEL was established at 200 ppm (27.35 and 38.96 mg/kg bw/day in males and females, respectively), based on decreased body weight/weight gain in males and an increased incidence of lung bronchiolization in males and females. The NOAEL was established at 30 ppm (4.2 and 6.4 mg/kg bw/day in males and females, respectively).

An increased incidence in nodules in the lung of females treated at 200 and 1200 ppm was recorded at terminal necropsy. The incidence of affected animals was 1, 6, 11 and 13 in the control, 30, 200 and 1200 ppm groups, respectively. Microscopically, the incidence of neoplastic lesions in the lungs was increased in females treated at 200 and 1200 ppm. The neoplastic lesions consisted of statistically significantly increased alveolar/bronchiolar adenomas in females treated at 200 and 1200 ppm, compared to control females (4, 8, 28 and 18%, control to high dose). The number of alveolar/bronchiolar carcinomas in females treated at 1200 ppm did not attain statistical significance using the Fisher's exact test, while it was positive with the Peto trend test (4, 2, 2 and 8%, control to high dose). The latency to tumor onset was also reduced for the carcinomas detected at 1200 ppm. The incidence of carcinoma, but not adenoma, was within historical control range. Combined tumor incidence was 8, 10, 30 and 26%, control to high dose (historical control values not provided). Males did not show a treatment-related increase.

Dosing was considered adequate based on decreased body weight/weight gain and an increased incidence of lung bronchiolization in both sexes.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for an oral carcinogenicity study in the mouse (OECD 451; OPPTS 870.4200).

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH
Description:	Solid, yellow
Lot/Batch:	36372130-291-PF1
Purity:	96.7-98.65%
CAS#:	318290-98-1
Stability:	Stable until May 2010
2. Vehicle	Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Mouse
Strain	CD-1 SPF
Age	Approximately 5 weeks at delivery
Weight	Males: 19.6 to 29.7 g; Females: 16.8 to 24.1 g at start of acclimation
Source	Charles River Germany, Sulzfeld, Germany
Acclimation period	6 days
Diet	Pelleted standard Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water	Community tap water from Itingen *ad libitum*
Housing	Individually in type 2 Makrolon cages with wire mesh tops and standardized softwood bedding (‘Lignocel’ Schill AG, Switzerland)
4. Environmental conditions	
Temperature	22 ± 3°C. Values outside of this range occurred occasionally, usually following room cleaning, and were considered not to have any influence on the study.
Humidity	30-70%. Values outside of this range occurred occasionally, usually following room cleaning, and were considered not to have any influence on the study.

Air change 10-15 air changes per hour

Photoperiod Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN

1. In-life dates 20 February 2008 to 4 September 2009 (treatment initiated 26 February)

2. Animal Assignment and treatment

The animals were assigned to groups using a computer-generated random algorithm (Table 1). After randomization three animals were exchanged with reserve animals to minimize differences in mean body weights between groups. Note that although 8 animals were assigned to the allocation B groups, only 6 animals underwent examination for liver enzymes.

Table 1: Study Design for a Carcinogenicity study in Mice

Allocation		Number of animals	0 ppm	30 ppm	200 ppm	1200 ppm
		Daily intake (mg/kg bw/day)				
Males	**A**	50	0	4.24	27.35	152.31
	B	8	0	nc	nc	nc
Females	**A**	50	0	6.43	38.96	188.44
	B	8	0	nc	nc	nc

Data obtained from pages 275 and 280 of the study report.
A: Oncogenicity animals; sacrificed at 78 weeks.
B: Animals for liver enzyme determinations; sacrificed at 13 weeks.
nc: daily intake not calculated separately for allocation B animals.

3. Dose Selection Rationale

Doses were selected based on the results of the 90-day dietary study in mice (study R-23151; MRID 4574753; PMRA 2181205). In that study, fluensulfone was administered in the diet at 0, 60, 300, and 1500 ppm (equivalent to average daily intakes of 0/0, 11/18, 51/68 and 228/252 mg/kg bw/day in males/females, respectively). Slight changes in hematology, blood biochemistry and food consumption were noted at 300 ppm, with more effects (e.g. decreased body weight, liver pathology) observed at 1500 ppm. The NOAEL was established at 60 ppm.

4. Diet Preparation and Analysis

Fresh batches of the feed pellets for the study were prepared weekly for the first two weeks, and then every two weeks thereafter.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40 °C until the test item was fluid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum approx. 40 °C). The test item was divided into three aliquots.

The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum of approximately 40 °C on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group. An appropriate amount of water was added to aid pelleting. The pellets were dried with air for approximately 24 to 96 hours before storage.

Control feed for the animals of group 1 was prepared similarly, but without test item.

Feed preparations were stored at room temperature (20±5 °C) in disposable paper bags until use. They were determined to be stable for up to 3 weeks in previous studies.

Concentration and homogeneity of the actual test item batch in the feed were determined before the start of treatment and at weeks 1, 3, 5, 7, 11, 25, 37, 51 and 63.

Analyses were performed using an HPLC-method, previously developed at the performing laboratory. The diet samples were stored deep-frozen (-20°C ± 5°C) until analysis. The test item was used as analytical standard.

5. Statistics

The following methods were used to analyze food consumption, body weight, clinical laboratory data, organ weights and ratios as well as macroscopic findings:

- If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups with control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- The Fisher’s exact test.

The statistical tests used for the analysis of microscopic findings are indicated in the relevant data tables in this review.

C. METHODS

1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once daily during acclimatization and once daily during the treatment period. Detailed clinical observations including palpation for tissue masses were

performed weekly during acclimatization and treatment periods.

2. Bodyweight

Body weights were recorded weekly during acclimatization and treatment weeks 1 to 14, and once every four weeks thereafter.

3. Food consumption

Food consumption was recorded weekly during acclimatization and treatment weeks 1 to 14, and once every four weeks thereafter.

4. Hematology and Clinical chemistry

Blood samples were drawn from the retro-orbital plexus, using a micro-hematocrit glass capillary tube, from all animals (allocation A) under light isoflurane anesthesia. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms at week 52 and after 78 weeks. At week 52, only hematology parameters (erythrocyte count, total and differential leukocyte count) were analyzed whereas after week 78 clinical biochemistry parameters (i.e. aspartate aminotransferase, alanine aminotransferase and sorbitol dehydrogenase) were determined from the 6 surviving animals with the lowest animal number of each group and sex. Hematology parameters were determined from the remaining animals at week 78.

Blood samples were also taken from a total of 13 moribund animals prior to sacrifice.

Blood smears were prepared for all animals (except those used for clinical biochemistry) at weeks 52 and 78.

5. Sacrifice and pathology

Allocation B animals were terminated after 13 weeks of treatment; allocation A animals after at least 78 weeks of treatment.

All animals found dead, moribund or sacrificed *in extremis* were necropsied.

All allocation B animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. The liver was sampled for liver enzyme determination (see below).

All allocation A animals were weighed and necropsied. All animals surviving to the end of the treatment period and all moribund animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. Descriptions of all macroscopic abnormalities were recorded.

Livers of all animals from which blood for clinical biochemistry was taken after week 78 were cut into two equal pieces. One piece was used for histopathology, the other was used for liver enzyme determination.

Samples of a complete list of tissues and organs were collected from all animals at necropsy and, unless otherwise indicated, fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but were not processed or examined further.

From allocation A animals, adrenal glands, brain, epididymides, heart including auricles, kidneys, liver (with gall bladder), ovaries, prostate gland, spleen, testes and uterus were weighed before fixation. Relative organ weights were calculated on the basis of the body weight and brain weight.

Slides of all organs and tissues of the control and high-dose groups and all gross lesions from all animals were examined by the study pathologist (including adrenal gland, aorta, bone, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerve, Harderian gland, heart, ileum, jejunum, kidneys, larynx, liver, lung, lymph nodes, mammary gland, nasal cavity, ovaries, pancreas, pharynx, pituitary gland, prostate gland, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina, all gross lesions and tissue masses). Organ and tissue samples taken from animals which died spontaneously or which were killed *in extremis* were evaluated similarly to those organs taken from animals of the high-dose group. Due to test item-related morphologic changes detected in the lungs of high-dose animals, lungs were also processed and examined from the mid- and low-dose group.

For investigation on the nature of the bronchiolization, the lungs of two control (nos. 33 and 253) and two high dose animals (nos. 218 and 419) were analyzed by transmission electron microscopy.

6. Liver Enzyme Determination

At scheduled necropsy after 13 weeks, the liver was sampled from all allocation B animals. After 78 weeks, half of the liver of all animals from which blood for clinical biochemistry was taken. Livers from the first 6 allocation B animals per sex and group, sampled after week 13, were homogenized, subcellular fractions were prepared and the following enzyme activities determined:

- Protein content of subcellular fractions
- Microsomal cytochrome P450 content (differential spectroscopy)
- CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation)
- CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation)
- CYP2B1 (microsomal 7-pentoxyresorufin O-dealkylation)

• CYP3A (microsomal testosterone 6β-hydroxylation)

• CYP4A1 (microsomal lauric acid 12-hydroxylation)

• Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)

• Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol)

• Microsomal epoxide hydrolase (hydrolysis of styrene oxide)

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

• Peroxisomal beta Oxidation

Portions of liver from 6 allocation A animals per sex and group, sampled after week 78, were homogenized, subcellular fractions were prepared and the following parameter was determined:

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

II. RESULTS AND DISCUSSION

A. ANALYSIS OF FEED PREPARATIONS

The identity of fluensulfone was confirmed by its retention time which was similar to that measured in the working standards. The test item content in 93 out of 99 samples met the required content limit of ±20% with reference to the nominal content. The three samples of the 30 ppm diet prepared on 21-Feb-2008 were slightly above 120% of the nominal concentration and the variation from the mean was 0.6%. The 30 ppm diet prepared on 28-Feb-2008 had 74%, 182% and 77% of the nominal content at the top, middle and bottom, respectively, with a mean recovery of 111% and maximum variation from the mean of 64%. The six samples outside the limit range were all from the first two dose preparations. In addition, the homogenous distribution of fluensulfone in the preparations is considered appropriate because the result of 32 out of 33 dose-preparations deviated by not more than 8.5% (<15%) from the corresponding mean.

The absence of test item in the control diet was confirmed.

In conclusion, the results obtained confirm the accurate preparation and use of fluensulfone and the control feed during the conduct of this study. Diet samples were found to contain acceptable concentrations and to be homogenously prepared.

Stability testing carried out in in the 2-year dietary study in the rat (Study Report R-23353), using the same test item and the same feed, showed that fluensulfone was stable in feed

preparation stored at 20 ± 5°C for at least three weeks.

B. OBSERVATIONS

1. Clinical signs

There were no treatment-related clinical signs.

No test-item-related increase in the incidence of palpable masses occurred.

The masses noted were not distinguishable between treated groups and controls with respect to their onset, incidence or location. Most frequently, small masses were recorded in males in the genital region.

2. Mortality

Mortality was not affected by treatment with fluensulfone (Table 2). The most common cause of death was amyloidosis, followed by systemic neoplasia; there was no dose-related increase in any particular cause of death.

Table 2: Mortality data (allocation A animals only) (N=50 per group)

Dose (ppm)	Spontaneous death		Killed *in extremis*		Planned necropsy	
	Males	Females	Males	Females	Males	Females
0	10 (20%)	6 (12%)	4 (8%)	1 (2%)	36 (72%)	43 (86%)
30	6 (12%)	9 (18%)	2 (4%)	4 (8%)	42 (84%)	37 (74%)
200	3 (6%)	12 (24%)	1 (2%)	2 (4%)	46 (92%)	36 (72%)
1200	8 (16%)	8 (16%)	1 (2%)	6 (12%)	41 (82%)	36 (72%)

Data obtained from page 33 of the study report.

C. BODYWEIGHT AND BODYWEIGHT GAINS

Mean body weight and body weight gain were decreased in a dose-dependent manner at 200 ppm (males only) and 1200 ppm. Males were more sensitive than females.

Mean body weight of males treated at 1200 ppm was statistically significantly reduced from test day 1 onwards (body weights were measured approximately 4 hours after treatment start on day 1) throughout the whole treatment period (from 3 to 20 % lower than controls). This may have been due to slightly lower body weights (3% below controls) in the 1200 ppm males at the start of the study. However, since mean cumulative body weight gain was also significantly reduced in both sexes (see below) and females also had lower body weights through a large part of the study, the changes in male body weights after the first few weeks were judged to have been due to treatment. Males treated at 200 ppm showed statistically significantly reduced mean body weight from week 5 (day 36) through week 54 (day 379) in the range of 3 to 8% from the control mean.

Mean body weight was also affected in females treated at 1200 ppm, where a statistically significant decrease could be observed in most measurements from week 2 onwards (with decreases of up to 12% relative to controls); all body weight measurements from week 38 to study termination were statistically significantly lower than controls.

The mean body weights at the end of the treatment period were reduced by 6% and 17% in the males of the mid and high dose groups, and by 12% in the females of the high dose group.

Body weight gain of males treated at 1200 ppm was statistically significantly reduced from week 4 onwards throughout the treatment period (18-47% lower than controls). In males treated at 200 ppm, body weight gain was also reduced (5-18% lower than controls), although not always achieving statistical significance.

In females, body weight gain was reduced at 1200 ppm only, and statistical significance was achieved for most time points from week 16 onwards (10-31% lower than controls).

Overall body weight gain in males at 200 ppm and 1200 ppm was 54g and 39g, respectively, compared to 62g in the controls (compared to starting body weight). Overall body weight gain in females at 1200 ppm was 49g, compared to 70g in the controls (compared to starting body weight). When compared to controls, the overall body weight gain in males from the 200 and 1200 ppm dose groups was decreased 13% and 37%, and the overall body weight gain in females from the 1200 ppm dose group was decreased 30% (Table 3).

Table 3: Selected mean ± SD body weights (g) and body weight gains (g)

Day	Control	30 ppm	200 ppm	1200 ppm
Males				
Initial BW	29.7 ± 1.4	29.3 ± 1.7	29.3 ± 1.5	28.7 ± 1.7** (↓3%)
BW Wk 5	36.0 ± 2.3	35.1 ± 2.2	34.7 ± 2.5** (↓4%)	33.2 ± 2.0** (↓8%)
BW Wk 54	47.6 ± 7.1	46.1 ± 6.6	44.0 ± 6.2* (↓8%)	37.9 ± 3.6** (↓20%)
Final BW	47.7 ± 5.9	48.3 ± 6.7	45.0 ± 6.4 (↓6%)	39.8 ± 4.2** (↓17%)
BWG Wk 1	6.7 ± 2.8	6.7 ± 3.6	6.7 ± 7.0	4.7 ± 4.8 (↓30%)
BWG Wk 1-13	35 ± 13	34 ± 13	30 ± 17	24 ± 7** (↓31%)
Overall BWG	62 ± 21	66 ± 23	54 ± 23 (↓13%)	39 ± 14** (↓37%)
Females				
Initial BW	21.8 ± 1.2	21.5 ± 1.2	21.7 ± 1.2	21.6 ± 1.3
Final BW	36.7 ± 5.0	36.6 ± 6.7	38.0 ± 6.2	32.3 ± 3.6** (↓12%)
BWG Wk 1	7.0 ± 4.3	6.9 ± 6.1	8.1 ± 3.8	4.9 ± 4.1* (↓30%)
BWG Wk 1-13	30 ± 11	32 ± 13	36 ± 12*	32 ± 8
Overall BWG	70 ± 25	70 ± 31	74 ± 26	49 ± 13** (↓30%)

Data obtained from pages 282-302 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

D. FOOD CONSUMPTION

Absolute food consumption (g/animal/day) was statistically significantly reduced at most weighing intervals in both sexes treated at 1200 ppm (data not presented). The overall mean was 17% lower in males and 24% lower in females, compared to controls.

In males, relative food consumption (g/kg bw/day) was not affected by treatment. However, in females relative food consumption was statistically significantly reduced at most weighing intervals at 1200 ppm and the overall mean over the treatment duration was reduced by 20% compared to controls.

E. HEMATOLOGY AND CLINICAL CHEMISTRY

After 52 weeks, hematology analysis revealed slightly decreased absolute red blood cell counts in mice of both sexes at 1200 ppm and decreased total white blood cells count in females only, accompanied by reductions in absolute neutrophil, eosinophil, basophil, lymphocyte (not statistically significant) and monocyte counts (Table 4). Absolute neutrophil and eosinophil counts were also statistically significantly reduced in females at 200 ppm. Non-statistically significant reductions in total white blood cells and in monocyte counts were noted in females from the 200 ppm group as well. None of these changes was still present at terminal sacrifice after 78 weeks.

Table 4. Mean hematology parameters

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Males - 52 Weeks				
RBC (10^{12}/L)	8.85	8.73	8.47	8.40 (↓5%)*
WBC (10^9/L)	6.79	6.15	5.46	6.92
Diff. WBC - Neutrophils (10^9/L)	2.28	1.50	1.40	2.14
- Eosinophils (10^9/L)	0.28	0.27	0.20	0.21
- Basophils (10^9/L)	0.04	0.03	0.02	0.03
- Lymphocytes (10^9/L)	3.86	4.09	3.63	4.27
- Monocytes (10^9/L)	0.12	0.14	0.13	0.11
- Leukocytes (10^9/L)	0.21	0.12	0.08	0.16
Males - 78 Weeks				
RBC (10^{12}/L)	8.58	8.25	8.14	8.41
WBC (10^9/L)	6.34	6.36	5.91	7.27
Diff. WBC - Neutrophils (10^9/L)	1.87	1.92	1.69	2.21
- Eosinophils (10^9/L)	0.27	0.23	0.18	0.26
- Basophils (10^9/L)	0.04	0.05	0.03	0.04
- Lymphocytes (10^9/L)	3.94	3.90	3.80	4.47
- Monocytes (10^9/L)	0.12	0.12	0.10	0.14
- Leukocytes (10^9/L)	0.10	0.15	0.12	0.14
Females – 52 Weeks				
RBC (10^{12}/L)	8.33	8.35	8.23	7.83** (↓6%)
WBC (10^9/L)	4.85	4.38	3.91 (↓19%)	3.56** (↓26%)
Diff. WBC - Neutrophils (10^9/L)	1.46	1.28	0.93 (↓36%)**	0.90** (↓38%)
- Eosinophils (10^9/L)	0.21	0.15	0.12 (↓43%)**	0.10** (↓52%)
- Basophils (10^9/L)	0.04	0.04	0.04	0.03** (↓25%)
- Lymphocytes (10^9/L)	2.95	2.73	2.65	2.40 (↓19%)
- Monocytes (10^9/L)	0.09	0.09	0.06 (↓33%)	0.06* (↓33%)
- Leukocytes (10^9/L)	0.11	0.09	0.12	0.08
Females - 78 Weeks				
RBC (10^{12}/L)	8.18	8.15	7.89	8.01
WBC (10^9/L)	4.30	4.30	4.65	4.54
Diff. WBC - Neutrophils (10^9/L)	1.51	1.46	1.58	1.42
- Eosinophils (10^9/L)	0.17	0.15	0.21	0.15
- Basophils (10^9/L)	0.04	0.04	0.04	0.03
- Lymphocytes (10^9/L)	2.41	2.46	2.64	2.75
- Monocytes (10^9/L)	0.09	0.09	0.08	0.09
- Leukocytes (10^9/L)	0.10	0.11	0.11	0.11

Data obtained from pages 305-308 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). Standard deviations were not provided in the study report.

* $p < 0.05$; ** $p < 0.01$

Marked increases were noted in sorbitol dehydrogenase (SDH) for males and females from the 200 and 1200 ppm dose groups, and in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) in females from the 200 and 1200 ppm dose groups when compared to controls (Table 5). These increases did not reach statistical significance nor were they of a magnitude that would be considered adverse.

Table 5. Mean clinical chemistry parameters at 78 weeks

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Males				
ASAT (U/L)	107.7	93.7	82.5	104.4
ALAT (U/L)	97.4	51.7	45.5	75.4
SDH (U/L)	9.0	11.2	21.1 (↑134%)	27.7 (↑208%)
Females				
ASAT (U/L)	98.7	96.9	132.0 (↑34%)	160.4 (↑62%)
ALAT (UL)	29.4	40.6	54.3 (↑85%)	61.5 (↑109%)
SDH (UL)	12.9	14.9	26.1 (↑102%)	24.8 (↑92%)

Data obtained from pages 310-311 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). Standard deviations were not provided in the study report. Historical control values – mean of 18 studies from April 2002 to November 2006 – ASAT in females from 71 weeks = 166.3 ± 136.3 U/L; ALAT in females from 71 weeks = 65.2 ± 53.3 U/L.

F. SACRIFICE AND PATHOLOGY

1. Organ Weights

In males treated with fluensulfone, dose-dependent, statistically significant decreases in absolute prostate weight, absolute brain weight and prostate to brain weight ratio were observed (Table 6a). In addition, a statistically significant decrease in absolute kidney weight, as well as a statistically significant increase in liver to body weight ratio, was recorded in females at 1200 ppm (Table 6b). These changes in organ weights did not correlate with any histopathological findings and were therefore not considered to be of toxicological relevance.

Statistically significantly increased organ to body weight ratios in males at 1200 ppm for brain, heart, kidneys, epididymides and testes were observed. A non-statistically significant increase in adrenal gland to body weight ratio was also observed in males at this dose. These findings are likely due to the reduced absolute body weight in this group, and in the absence of histological correlates, are not considered to be of any toxicological relevance.

Reductions in spleen weight (absolute, relative to body weight and relative to brain weight)

were also noted in males from the 1200 ppm dose group when compared to controls. These changes did not reach statistical significance, and there was a high degree of variability in spleen weight measurements in all dose groups. Furthermore, there were no pathological correlates indicating a toxic effect on the spleen, and the incidence of enlarged spleen was decreased in males in the 200 and 1200 ppm dose groups (incidence of 10, 10, 5, and 6 in the 0, 30, 200 and 1200 ppm dose groups, respectively). Therefore, these changes in spleen weight are not considered to be related to treatment.

Table 6a. Selected mean (± SD) absolute and relative organ weights in male mice

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Final body weight (g)	45.9 ± 6.1	46.1 ± 6.1	43.3 ± 6.0	38.8 ± 4.0** (↓15%)
Brain - absolute (g)	0.515 ± 0.020	0.508 ± 0.025	0.507 ± 0.025	0.501 ± 0.023* (↓3%)
- rel. to bw (%)	1.142 ± 0.157	1.119 ± 0.145	1.193 ± 0.163	1.303 ± 0.132** (↑14%)
Heart - absolute (g)	0.248 ± 0.024	0.255 ± 0.031	0.251 ± 0.034	0.234 ± 0.030 (↓6%)
- rel. to bw (%)	0.545 ± 0.063	0.559 ± 0.071	0.591 ± 0.141	0.607 ± 0.078* (↑11%)
Liver - absolute (g)	2.34 ± 0.72	2.25 ± 0.54	2.15 ± □.35	2.14 ± 0.30
- rel. to bw (%)	5.15 ± 1.55	4.94 ± 1.22	4.98 ± 0.63	5.53 ± 0.52
Kidney - absolute (g)	0.794 ± 0.157	0.796 ± 0.165	0.761 ± 0.129	0.740 ± 0.090
- rel. to bw (%)	1.740 ± 0.303	1.747 ± 0.387	1.772 ± 0.289	1.915 ± 0.192 (↑10%)*
Adrenals - absolute (g)	0.007 ± 0.002	0.007 ± 0.003	0.007 ± 0.002	0.009 ± 0.011
- rel. to bw (%)	0.015 ± 0.005	0.016 ± 0.006	0.015 ± 0.006	0.023 ± 0.029 (↑53%)
Spleen - absolute (g)	0.167 ± 0.202	0.168 ± 0.138	0.139 ± 0.111	0.112 ± 0.048 (↓33%)
- rel. to bw (%)	0.368 ± 0.415	0.371 ± 0.294	0.323 ± 0.278	0.291 ± 0.126 (↓21%)
- rel. to brain wt (%)	32.8 ± 41.1	33.4 ± 28.0	27.3 ± 21.4	22.6 ± 10.1 (↓31%)
Epidid. - absolute (g)	0.136 ± 0.017	0.134 ± 0.024	0.130 ± 0.019	0.132 ± 0.017
- rel. to bw (%)	0.300 ± 0.045	0.292 ± 0.046	0.302 ± 0.046	0.342 ± 0.048** (↑14%)
Testes - absolute (g)	0.239 ± 0.032	0.229 ± 0.035	0.219 ± 0.038	0.239 ± 0.048
- rel. to bw (%)	0.528 ± 0.097	0.504 ± 0.095	0.515 ± 0.105	0.623 ± 0.140** (↑18%)
Prostate - absolute (g)	0.125 ± 0.032	0.119 ± 0.023	0.106 ± 0.025** (↓15%)	0.098 ± 0.018** (↓22%)
- rel. to bw (%)	0.275 ± 0.072	0.260 ± 0.051	0.246 ± 0.055 (↓10%)	0.256 ± 0.052 (↓7%)
- rel. to brain wt (%)	24.2 ± 5.9	23.4 ± 4.5	20.9 ± 5.2** (↓14%)	19.7 ± 3.4** (↓19%)

Data obtained from pages 313-318 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

Table 6b. Selected mean (± SD) absolute and relative organ weights in female mice

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Final body weight (g)	34.7 ± 4.2	34.6 ± 6.6	35.7 ± 5.5	31.6 ± 3.7*
Brain - absolute (g)	0.527 ± 0.034	0.537 ± 0.048	0.533 ± 0.032	0.509 ± 0.027
- rel. to bw (%)	1.540 ± 0.198	1.604 ± 0.343	1.530 ± 0.263	1.631 ± 0.189
Heart - absolute (g)	0.197 ± 0.028	0.200 ± 0.023	0.199 ± 0.021	0.185 ± 0.020
- rel. to bw (%)	0.571 ± 0.076	0.594 ± 0.104	0.571 ± 0.124	0.588 ± 0.052
Liver - absolute (g)	1.75 ± 0.42	1.71 ± 0.35	1.78 ± 0.33	1.79 ± 0.37
- rel. to bw (%)	5.06 ± 1.06	4.99 ± 0.75	5.01 ± 0.70	5.62 ± 0.69** (↑11%)
Kidneys - absolute (g)	0.487 ± 0.085	0.469 ± 0.051	0.471 ± 0.057	0.442 ± 0.065** (↓9%)
- rel. to bw (%)	1.413 ± 0.234	1.384 ± 0.197	1.341 ± 0.201	1.403 ± 0.134
Adrenals - absolute (g)	0.012 ± 0.003	0.012 ± 0.003	0.012 ± 0.003	0.011 ± 0.002
- rel. to bw (%)	0.034 ± 0.008	0.035 ± 0.009	0.034 ± 0.009	0.036 ± 0.008
Spleen - absolute (g)	0.188 ± 0.127	0.219 ± 0.158	0.179 ± 0.091	0.217 ± 0.318
- rel. to bw (%)	0.546 ± 0.372	0.628 ± 0.422	0.524 ± 0.322	0.654 ± 0.828
Uterus - absolute (g)	0.696 ± 0.480	0.715 ± 0.510	0.785 ± 0.649	0.552 ± 0.367
- rel. to bw (%)	2.073 ± 1.524	2.113 ± 1.427	2.317 ± 2.157	1.724 ± 1.018
Ovaries - absolute (g)	0.285 ± 0.428	0.365 ± 0.558	0.239 ± 0.860	0.488 ± 1.338
- rel. to bw (%)	0.851 ± 1.322	1.065 ± 1.602	0.703 ± 2.583	1.300 ± 3.190

Data obtained from pages 319-321 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

2. Gross and Histopathology

Macroscopically, an increase in nodules in the lung of females at 200 and 1200 ppm was recorded. The incidence was 1, 6, 11** and 13** in the 0, 30, 200 and 1200 ppm groups, respectively (**: $p<0.01$; N=50).

At microscopic examination both non-neoplastic and neoplastic lesions were observed in the lungs of treated animals.

Non-neoplastic

An increase in the incidence and severity of bronchiolization was seen in the lung of males and females at 200 and 1200 ppm. Morphologically this finding mainly consisted of a change from flattened epithelium to cuboidal epithelium, or hypertrophy of the epithelium (Clara cells), lining the terminal bronchioles. In the highest dose, this change extended to the adjacent alveolar walls. The diagnosis was confirmed by using transmission electron microscopy (TEM) analysis which revealed hypertrophy of the epithelium of the terminal bronchioles affecting mostly the non-ciliated Clara cells as well as the surrounding ciliated

cells. These cells were arranged in few layers giving rise to a pseudo-stratified epithelium extending occasionally to the respiratory bronchioles and alveolar ducts.

Table 7: Incidence / mean severity grade of bronchiolization in lungs

	Male				Female[##]			
Dose (ppm)	**0**	**30**	**200**	**1200**	**0**	**30**	**200**	**1200**
No. examined	50	50	50	50	50	50	50	50
Bronchiolization	1	0	24**	31**	5	7	43**	48**
Mean severity grade	1.0	-	1.3	1.6	1.0	1.0	1.8	2.6

Data obtained from page 38 of the study report.
[##] $p<0.05$ using Armitage Trend Test
** $p<0.01$ using Fisher's Exact Test (One-Sided)

The remaining non-neoplastic findings recorded were within the range of normal background lesions observed in animals of the used strain and age.

Neoplastic

In the lungs, alveolar/bronchiolar adenomas were increased in females at 200 and 1200 ppm; although statistically significant by the Fisher's exact test, a clear dose-relationship was not evident. The incidence in females at 200 ppm and in both sexes at 1200 ppm exceeded the laboratory maximum historical control incidence of 14% in males and 6% in females. Alveolar/bronchiolar carcinomas in females at 1200 ppm were statistically significantly positive by the Peto trend test but not by the Fisher's exact test. Their incidence was slightly below the maximum historical control incidence reported for this strain of mice in the performing laboratory (up to 10% reported in control females versus 8% seen at 1200 ppm). In males, no statistically significant increases in tumors were observed. The incidence of alveolar/bronchiolar adenomas was outside the historical control range at 30 and 1200 ppm. Males did not show an increased incidence of carcinoma with treatment, but levels in controls were high compared to historical controls.

The onset (chronological occurrence) of adenomas and carcinomas in the female mice was reduced at 200 and 1200 ppm (Table 9), when compared to controls and females at 30 ppm, with the earliest onset observed at 1200 ppm (from week 50 on). In males there were no differences among control and treated groups in the onset of lung tumors.

Control females had a comparatively low incidence of both adenomas and carcinomas compared to control males, but the historical background incidence of each tumor type was also lower in females than in males. The combined incidence of adenoma and carcinoma was increased at 200 and 1200 ppm. In treated males, the combined incidence of

alveolar/bronchiolar adenomas and carcinomas was comparable between control and high dose animals and the incidence of each tumor type was also high among all dose groups including controls, relative to historical control values.

Table 8: Incidence of alveolar/bronchiolar tumors

	Males					Females				
Dose (ppm)	**0**	**30**	**200**	**1200**	**HC Data**	**0**	**30**	**200**	**1200**	**HC Data**
No. examined	50	50	50	50	266	50	50	50	50	265
Adenoma	7 (14%)	9	5	12 (24%)	18 (7%) (0-14%)	2 (4%)	4	14** (28%)	9* (18%)	9 (3%) (0-6%)
Carcinoma	8 (16%)	3	3	4 (8%)	22 (8%) (4-12%)	2 (4%)	1	1	4[#] (8%)	9 (3%) (0-10%)
Adenoma & carcinoma combined	15 (30%)	12	8	16 (32%)	Not given	4 (8%)	5	15	13[#] (26%)	Not given

Data obtained from pages 39 and 2469 of the study report.
*/** $p<0.05$ and $p<0.01$, respectively, using Fisher's Exact Test (one-sided and pairwise);
[#] $p<0.05$ using Peto Trend Test

Table 9. Individual onset (by study week) of alveolar/bronchiolar tumors

Tumor type	0 ppm	30 ppm	200 ppm	1200 ppm
Males				
Adenoma	62, 74, 79 (2), 80 (3)	52, 79 (6), 80 (2)	46, 80 (4)	73, 79 (4), 80 (7)
Carcinoma	62, 77, 79, 80 (5)	79 (2), 80	46, 79, 80	79 (3), 80
Median week onset	77	77	71	79
Females				
Adenoma	79 (2)	79 (2), 80 (2)	67, 76 (2), 79 (8), 80 (3)	56, 66, 70, 79 (3), 80 (3)
Carcinoma	79 (2)	79	79	50, 66, 79, 80
Median week onset	79	79	78	73

Data obtained from page 1327 of the study report. Numbers in parentheses represent the number of decedent or sacrificed animals with tumor onset for that week.

With the Peto trend test, a few other tumors attained a level of statistical significance; however considering their single or very low occurrence they were not considered to be of any

toxicological significance. A hemangiosarcoma of the ovaries (n=1) and uterus (n=1) was seen in individual females at 1200 ppm. However considering the very low incidence, and as stated by the study authors that hemangiosarcomas are rather common tumors in mice, it was regarded as a spontaneous occurrence and not related to the treatment. A higher incidence of hepatocellular adenomas was seen in males at 30 (n=7) and 200 ppm (n=6). The incidence of this benign tumor at 1200 ppm (n=1) was lower than in controls (n=3). Therefore, considering the absence of a dose response, they were regarded as incidental.

Some other tumors occurred in several organs/systems, but did not attain statistical significance and all were within the range of the biological variation that occurs in mice of this age. The organs most commonly affected by neoplasia were the hemolymphoreticular system, liver, Harderian gland and pituitary gland (females). In male mice, the incidence of malignant lymphomas was decreased at 200 and 1200 ppm when compared to controls. However the decrease, affecting males only, was within the biological variation reported for this strain of mice (Charles River monography on spontaneous neoplastic lesions in the Crl:CD1 (ICR) mouse, March 2010).

All remaining organs were infrequently affected by neoplasia and tumors were randomly distributed among control and treated groups. Some rare tumors were observed as single occurrences, namely a brain oligodendroglioma in a control female, a hemangioma in the testis of a control male, a rectal adenocarcinoma in a single high dose female, and a tubular cell carcinoma of the kidney in single males of the 0, 200 and 1200 ppm dose groups. However none of them was considered related to treatment.

G. LIVER ENZYME DETERMINATION

Fluensulfone caused a slight inhibition of hepatic ALAT activity in females treated at 1200 ppm for 13 weeks, but not for 78 weeks (Table 10). In addition, a weak increase of microsomal cytochrome P450 content, accompanied by slight increases of the activity of CYP4A-1 (lauric acid 12-hydroxylase) and CYP1A1 (7-ethoxyresorufin O-dealkylation) were observed in females only at 200 and 1200 ppm. The phase II enzymes uridine diphosphoglucuronosyl-transferase, glutathione S-transferase and epoxide hydrolase were slightly to moderately induced, with females being more susceptible than males.

There was no dose-related change in the activity of microsomal 7-methoxyresorufin O-dealkylase, 7-pentoxyresorufin O-dealkylase, 6 ß-testosterone hydroxylase, or peroxisomal ß-oxidation.

The results exclude a potent induction of liver enzymes by fluensulfone. Increases in a few phase I activities, observed in females only and mainly at the highest dose level, indicate that fluensulfone exerts a minor non-adverse activity, and no peroxisome proliferation can be

expected. Induction of some phase II activities, observed in both sexes generally at the highest dose level, can be considered an adaptive response to the constant load on the liver from a xenobiotic, Therefore, these effects were considered not to be adverse.

Table 10: Selected liver enzyme activities (Mean ± SD; % difference from control)

Parameter	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	F	M	F	M	F	M	F
ALAT (U/g) Week 13	177 ± 29	236 ± 57	207 ± 28	218 ± 15	187 ± 28	211 ± 31	177 ± 20	**126 ± 16** (↓47%)**
ALAT (U/g) Week 78	136 ± 25	160 ± 30	148 ± 18	151 ± 13	165 ± 24	161 ± 17	135 ± 29	136 ± 29
P450 (nmol/g)	26.2 ± 6	16.7 ± 3	25.6 ± 3	17.7 ± 2	28.1 ± 5	**21.7 ± 4* (↑30%)**	29.9 ± 3	**22.8 ± 3** (↑37%)**
EROD (nmol/min/g)	2.5 ± 0.6	1.6 ± 0.4	2.3 ± 0.5	1.9 ± 0.6	2.8 ± 0.6	**2.1 ± 0.9 (↑36%)**	3.1 ± 1.0	**2.6 ± 0.4* (↑63%)**
LA12OH (nmol/min/g)	12.6 ± 3	9.2 ± 3	14.4 ± 4	10.6 ± 4	10.1 ± 3	**15.6 ± 5 (↑68%)**	15.0 ± 5	**20.1 ± 6* (↑117%)**
GST (nmol/min/g)	288 ± 51	92.5 ± 15	300 ± 30	87.6 ± 23	323 ± 46	**145 ± 39* (↑57%)**	**440 ± 35** (↑53%)**	**368 ±101** (↑298%)**
UDPGT (µmol/min/g)	1.2 ± 0.4	1.1 ± 0.4	1.3 ± 0.2	1.1 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	**1.6 ± 0.2* (↑45%)**
mEH (nmol/min/g)	144 ± 20	62 ± 12	161 ± 41	**91 ± 24* (↑48%)**	**213 ± 42** (↑48%)**	**131 ± 31** (↑113%)**	**297 ± 34** (↑106%)**	**249 ± 36** (↑304%)**

Data obtained from pages 2503-2508 of the study report.
M: males; F: females; ALAT: alanine aminotransferase in 100g supernatants; P450: microsomal cytochrome P450 content; EROD: microsomal 7-ethoxyresorufin O-dealkylase; LA12OH: microsomal lauric acid 12-hydroxylase activity; GST: cytosolic glutathione S-transferase activity; UDPGT: microsomal uridine diphospho-glucuronosyl transferase activity; mEH: microsomal epoxide hydrolase activity
* and **: significantly different from control (Dunnett's t-test) at 5% and 1%, respectively

III. CONCLUSIONS

Due to effects on body weights in both sexes as well as neoplastic and non-neoplastic lesions in the lungs of females at 200 and 1200 ppm, the NOAEL was established at 30 ppm (4.2 and 6.4 mg/kg bw/day in males and females, respectively).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: Overall, the regulatory authorities agree with the majority of the conclusions reached by the study investigators and concur with the systemic toxicity LOAEL and NOAEL of 1200 and 200 ppm, respectively.

The study investigators concluded that the increase in alveolar/bronchiolar carcinomas in females from the 1200 ppm dose group were not treatment-related since the incidence fell within the range of control incidence in previous oncogenicity studies. However, due to the significant increase in adenoma exceeding historical controls at 200 and 1200 ppm, the statistically significant trend noted for carcinomas as well as for adenomas and carcinomas combined, and the shortened latency to onset for carcinomas at 1200 ppm in females, the relationship between the increased incidence of these tumors and exposure to fluensulfone cannot be ruled out.

OCS notes that while the onset of tumor formation in female CD-1 mice at 1200 ppm was reduced (largely due to onset of an adenoma at 56 weeks [i.e. in 1/9 animals] and a carcinoma at 50 and 66 weeks [i.e. in 2/4 animals]) the incidence of carcinoma was still within the historical control range. Also, although the incidence of adenomas in females was outside the historical control range at 200 and 1200 ppm (28% and 18% respectively) and statistically significant by the Fisher exact test a dose response relationship was not evident. Overall, OCS considers that based on the available data, fluensulfone exhibited a weak carcinogenic potential in female mice.

C. Conclusions: This carcinogenicity study in the mouse is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a carcinogenicity study (OPPTS 870.4200; OECD 451) in mice.

D. Deficiencies: Standard deviations for hematology and clinical chemistry analyses were not provided in the study report. This omission does not alter the conclusions or acceptability of the study. No other deficiencies were noted.